IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	:	10/551,565
Applicant	:	Edward L.G. Pryzdial et al.
TC/A.U.	:	1654
Confirmation No.	:	4718
Examiner	:	Christiana Marchetti Bradley
Docket No.	:	01826-57320

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION

Sir/Madam:

I, Edward Pryzdial, do hereby solemnly declare that:

(1) I am a citizen of Canada and am employed by Canadian Blood Services in Vancouver, Canada. A copy of my C.V. is enclosed in Appendix A.

(2) I am the one of the co-inventor of United States Patent Application Serial Number 10/551,565 filed on February 8, 2006. I am also one of the co-authors of the Grundy et al. publication (*Biochem.*, 2001, *40*, 6293-6302). I have read and am thoroughly familiar with the contents of the above-identified U.S. Patent Application, including the claims presently on file.

(3) I have also read and understood the latest Official Action from the PTO dated July 24, 2008. In this Office Action, claims 1, 3 to 6, 10, 11, 14 and 17 have been rejected as being allegedly obvious over Grundy et al. in view of Gladstone et al. Further, claims 1, 3 to 6, 10, 11, 15 and 16 have been rejected as being allegedly obvious over Grundy et al. in view of Llevladot et al.

(4) It is understood that the Examiner is taking the position that the Grundy et al. publication teaches that Factor Xaγ accelerates tissue plasminogen activator (e.g. tPA). Since tPA is already known to be useful in the treatment of stroke thrombosis (as exemplified by Glastone et al.) and myocardial infarction thrombosis (as suggested by Llevadot et al.), the Examiner concludes that the prior art teaches the use of Factor Xaγ to accelerate clot dissolution in individuals afflicted with thrombosis.

(5) The results presented in Grundy et al. do not show that Factor Xaγ is a tPA accelerator and consequently, that it could be successfully used to accelerate blood clot dissolution in a subject in need thereof. In the art, it is known that binding assays (such as those presented in Grundy) are not a reliable predictor of coagulation protein function. In the context of tPA accelerators, the reason is that the accepted gold standard physiological tPA accelerator is the clot itself (i.e. fibrin). The clot is in vast excess over Factor Xaγ. Therefore, according to current art, the clot would logically overwhelm any effects of Factor Xaγ.

(6) The technology developed by Grundy et al. was reviewed by anonymous peers in a grant application (Appendix B) and prior to its publication (Appendix C). Both of these documents concluded that the *in vitro* results presented in Grundy et al. cannot be used to predict that Factor Xaγ can be used as a tPA accelerator in the context of the clot. In the grant review (Appendix B), it was clearly indicated that:

> "Since the concentration of FV or even that of FX in plasma is at least an order of magnitude less than the concentration of fibrinogen (fibrin), a ratio which would probably be maintained within a clot, it is difficult to envisage a scenario where components of prothrombinase will be cleaved by plasmin in sufficient quantity to compete with fibrin (...) to stimulate tPA-dependent activation of plasminogen.""

In the manuscript review (Appendix C), it is indicated that.

"In addition, I wonder whether or not the stimulatory potential of degraded FX(a) on tPA-induced plasminogen activation is relevant in the presence of a great stimulatory potential of intact fibrin..."

(7) Contrary to what is currently known in the art, the clot-dissolving experiments presented in the present patent application unambiguously indicate that the clot does not overwhelm effects mediated by Factor Xaγ and consequently, Factor Xaγ may be successfully used to accelerate blood clot dissolution in a subject in need thereof. These experiments provide a reliable prediction that Factor Xaγ will enhance solubilization of the clot *in vivo* in comparison to the experiments published in Grundy et al., which merely evaluated binding between Factor Xaγ and tPA or plasminogen.

(8) Further, it is not scientifically sound to predict that any protein that binds tPA or plasminogen *in vitro* will accelerate tPA and clot dissolution. For reasons already cited, the clot is anticipated to overwhelm the effects of other tPA- or plasminogen-binding proteins. Furthermore, the binding protein is believed to require association with both simultaneously to bring the tPA (enzyme) and plasminogen (substrate) into close proximity. This was never shown for Factor Xaγ.

(9) Other than fibrin or fragments of fibrin, I am not aware of published data showing that a protein able to bind either tPA or plasminogen can directly accelerate tPA *in vivo* or in plasma. Many binding-proteins have been identified that have been shown to interact with either tPA or plasminogen, for example: antithrombin III (Dudani, *Thromb Res.*, 2000, *99*, 635-41), tetranectin (Heilskov et al., *J. Biol. Chem.*, 1998, *273*, 29241-46), fibronectin (Salonen et al., *J Biol Chem.*, 1985, *260*, 12302-7.), alpha-enolase (Miles et al., *Biochemistry*, 1991, *30*,1682-91), osteonectin and annexin 2 (Hajjar et al., *J Biol Chem.*, 1996, *271*, 21652-9). Of these, to my knowledge, only annexin 2 has been reported to conclusively accelerate tPA to generate plasmin from plasminogen. In complete contradiction to the premise that tPA acceleration and the resulting

plasmin formation must result in enhanced clot lysis, annexin 2 was shown to inhibit rather than speed-up fibrin dissolution *in vitro* (Choi et al., *Biochemistry*, 1998, *37*, 648-55). Further complicating the picture, when annexin 2 was genetically deleted from mice, clot clearance was apparently prolonged (Ling et al., *J Clin Invest.*, 2004, *113*, 38-48). The latter was not shown to be a direct effect on tPA and may be the result of cell signaling (Battachargee et al., *Circ. Res.*, 2008, *102*, 457-464).

(10) Accordingly, it is currently known in the art that *in vitro* binding studies of coagulation factor cannot soundly predict the acceleration of tPA and/or enhancement of fibrinolysis. Therefore, a person skilled in the art will acknowledge that the methods disclosed in the present application are not obvious having regard to Grundy et al.

(11) I, the undersigned, declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C §1001 of the United States Code and that such willful false statements may jeopardize the validity of any patent issued for the above-referenced patent application.

By: _____ Date: 2008-11-19

Edward Pryzdial

APPENDIX A

EDUCATION

1981-1987
Ph.D., University of Toronto
Research Supervisor: Dr. David E. Isenman, Complement protein biochemistry

1977-1981
Hon.B.Sc., University of Toronto
Research Supervisor: Dr. Raymond Cummins, Arctic plant adaptation physiology

PROFESSIONAL TRAINING

1987-1990
Medical Research Council of Canada, Postdoctoral Fellow
NIH Specialized Center of Research in Thrombosis, University of Vermont
Research Supervisor: Dr. Kenneth G. Mann, Coagulation protein biochemistry

POSITIONS HELD

1998-Present
Scientist
Research and Development Department
Canadian Blood Services

2008-Present
Clinical Professor
Department of Pathology and Laboratory Medicine
University of British Columbia

2001-2008
Clinical Associate Professor
Department of Pathology and Laboratory Medicine
University of British Columbia

2004-Present
Faculty
University of British Columbia,
Centre for Blood Research
Life Sciences Institute

1990-1998
Research Scientist
Research Department
The Canadian Red Cross Society

1992-2003
Adjunct Professor,
Department of Biochemistry, Microbiology and Immunology, University of Ottawa

SCHOLARLY AND PROFESSIONAL ACTIVITIES

Current Grants Held

Granting Agency	Subject	Year	Principal Investigator	Co-Investigator(s)
HSF BC/Y	Effect of plasmin on prothrombinase	2007-2010	E. Pryzdial	
HSF BC/Y	Coagulation initiated on herpes viruses	2006-2009	E. Pryzdial	
Canadian Blood Services	Profiling hemostasis serine hydrolases in plasma	2007-2009	E. Pryzdial	R. Kainthan, D. Brooks
CIHR Strategic Training Initiative	Transfusion Science Training Program	2003-2009	R. MacGillivray	E. Pryzdial and others
MSFHR Infrastructure Funding Program	Center for Blood Research and Laboratory for Molecular Biophysics	2004-2008	R. MacGillivray	E. Pryzdial and others

Invited Presentations

1 University of Western Ontario, Dept. of Biochemistry, 1993
2 McMaster University, Vascular Biology Research Group, 1999
3 University of British Columbia, Dept. of Biochemistry, 2000
4 Queen's University, Dept. of Biochemistry, 2000

5 Ottawa Heart Institute, 2001

6 St. Paul's Hospital, iCapture, 2002

7 Children's Hospital of Philadelphia, Stokes Research Institute, 2002

8 Emory University, Atlanta, Georgia, 2005

9 University of Western Ontario, Dept. of Biochemistry, 2006

10 University of Toronto, 2007

11 McMaster University, 2007

12 FASEB Meeting on Proteases in Hemostasis and Vascular Biology, 2007

13 Centre for Drug Research and Development, 2008

14 Puget Sound Blood Bank, 2008

15 Oregon Health and Science University, 2008

Other Presentations

1 Derry, M.C., Raynor, C.M., Waisman, D.M. and Pryzdial, E.L.G. (2003) Annexin II enhances cytomegalovirus Infection. International Annexin Workshop

2 Sutherland, M.R., Friedman, H.R. and Pryzdial, E.L.G. (2002) Thrombin Enhances Herpesvirus Infection. American Society of Hematology

3 Sutherland, M.R., Gillies, C., Friedman, H., Cohen, G., Eisenberg, R. and Pryzdial, E.L.G. (2000) Herpes Simplex Virus Glycoprotein C Links Infection to Coagulation, American Society of Blood Bankers

4 Pryzdial, E.L.G., Raynor, C.M., Wright, J.F. and Waisman, D.M. (1999) Annexin II enhances cytomegalovirus binding and fusion to phospholipid membranes. American Society of Virology

5 Pryzdial, E.L.G. (1995) Feedback conversion of prothrombinase into a tissue plasminogen activator-accelerator and plasminogen receptor. International Society of Thrombosis and Haemostasis

Conference Participation (Organizer, Keynote Speaker, etc.)

2008 International Society of Thrombosis and Haemostasis, Scientific Advisory Committee

2007 International Society of Thrombosis and Haemostasis, Abstract Review Committee

2004 American Society of Hematology, Oral Session Moderator

2007 International Society of Thrombosis and Haemostasis, Abstract Review Committee

2003 American Society of Hematology, Abstract Review Committee

2002-2003 Frontiers in Cardiovascular Science Conference Organizing Committee

2003 Frontiers in Cardiovascular Science Conference Panellist

2001-2002 International Society of Blood Transfusion (ISBT), Scientific Review Committee

2002 ISBT Symposium Chair, Protein Products I

2002 ISBT Symposium Chair, Protein Products II

2002 ISBT Symposium Chair, Pathogen Invasion Mechanisms

2002 ISBT Symposium Chair, Cell and Protein Biology Review

SERVICE TO THE UNIVERSITY

Memberships on committees, including offices held and dates

2002-present UBC/Pathology, Research Committee
2002-present UBC/Pathology, Graduate Advisory Student Committee
2003-present UBC, Faculty of Medicine, Scholarship Review Committee
2003-present Centre for Blood Research, Scholarship Review Committee, Chair

Other service, including dates

2004-present UBC Centre for Blood Research, Training Program Member
2004-present UBC Centre for Blood Research, Training Program Application Review Committee
2003-present UBC Centre for Blood Research, Seminar Program Chair
2005-present UBC Centre for Blood Research, Executive Steering Committee
2005-present LSC 4th floor Safety Committee

SERVICE TO SCIENTIFIC COMMUNITY

Memberships on scholarly societies, including offices held and dates

American Association of Blood Banks, Member

American Society of Virology, Member

American Chemical Society, Biochemistry Division, Member

Canadian Federation of Biological Sciences, Member

American Society of Hematology, Member

Canadian Society of Transfusion Medicine, Member

Memberships on scholarly committees,

1999-2000 CBS Compensation Review Committee, Member

1999-present Canadian Blood Services R&D Working Group, Chair

1999-present Canadian Blood Services Intellectual Property Working Group, Member

2000-present Canadian Blood Services Communications Working Group, Member

REVIEWER

Grant Committees

2003-2005 CIHR Scientific Review Committee, Experimental Medicine, Member

2003-present Heart and Stroke Foundation of BC and Yukon, Research Advisory Committee

2003 CIHR Scientific Review Committee, Experimental Medicine, Invited Member

2002 CIHR Scientific Review Committee, Experimental Medicine, Invited Member

1998-2001 Heart and Stroke Foundation of Canada, Scientific Review Committee

Journal Board

2003-2006 Annexins, Editorial Board

Ad Hoc Grant Reviewer

Canadian Institutes of Health Research

Heart and Stroke Foundation of Canada

Welcome Trust, United Kingdom

Ad Hoc Manuscript Reviewer

Biochemical Journal

Biochemistry

Blood

Cytometry

Journal of Chromatography

Journal of Thrombosis and Haemostasis

Oncogene

Thrombosis and Haemostasis

Thrombosis Research

Transfusion

AWARDS

1987 Medical Research Council of Canada Postdoctoral Fellowship

1991 American Heart Association Young Investigator Travel Stipend

PUBLICATIONS

Manuscripts Submitted:

1) Song, J., Talbot, K., Hewitt, J., MacGillivray, R.T.A. and Pryzdial, E.L.G. (2008) Differential contribution of Glu96, Asp102 and Asp111 to coagulation factor Va subunit stability. Biochemical Journal
2) Talbot, K., Meixner, S.C. and **Pryzdial E.L.G.** (2008) Coagulation factor Xa derivatives, Xa33/13 and FXa, enhance plasminogen activation and fibrin clot lysis. Biochemical Journal

Reviews:

1) Krishnaswamy, S., Nesheim, M.E., **Pryzdial, E.L.G.** and Mann, K.G. (1993) Assembly of prothrombinase complex. Methods in Enzymology 222:260-280

Papers in Refereed Journals:

1) Churg, A., Wang, X., Wang, D., Meixner, C.M., **Pryzdial, E.L.G.** and Wright. J.L. (2007) α-1-antitrypsin suppresses release of TNFα from cigarette smoke-stimulated alveolar macrophages. American Journal of Respiratory Cell and Molecular Biology 37:144-151

2) Sutherland, M.R., Friedman, H.M. and **Pryzdial, E.L.G.** (2007) Thrombin production initiated by herpes simplex virus increases infection of cells through protease activated receptor 1. Journal of Thrombosis and Haemostasis 5:1055-1061

3) Grundy, J.E., Hancock, M. MacKenzie, C.R., Koschinsky, M., and **Pryzdial, E.L.G.** (2007) Plasminogen kringles 1-3 mediate C-terminal Lys-dependent

and -independent binding to plasmin-modulated factor Xa. Thrombosis and Haemostasis 97:38-44.

4) Derry, M.C., Sutherland, M.R., Restall, C.M., Waisman, D.M. and **Pryzdial, E.L.G.** (2007) Annexin 2-mediated enhancement of cytomegalovirus infection opposes inhibition by annexin 1 or annexin 5. Journal of General Virology 88:19-27

5) Livingston, J.R., Sutherland, M.R., Friedman, H.M. and **Pryzdial, E.L.G.** (2006) Herpes simplex virus type 1-encoded glycoprotein C contributes to direct coagulation factor X-virus binding. Biochemical Journal 393: 529-535

6) Sutherland, M.R., Friedman, H.M., **Pryzdial, E.L.G.** (2004) Herpes simplex virus type 1-encoded glycoprotein C enhances coagulation factor VIIa activity on the virus. Thrombosis and Haemostasis 92: 947-955

7) Zeibdawi, A., Grundy, J.E., Lasia, B. and **Pryzdial, E.L.G.** (2004) Coagulation factor Va Glu96-Asp111: A chelator-sensitive site involved in function and subunit association. Biochemical Journal 377:141-148

8) Peterson, E.A. ,Sutherland, M.R., Nesheim, M.E. and **Pryzdial, E.L.G.** (2003) Thrombin induces cell surface exposure of the plasminogen receptor, annexin 2. Journal of Cell Science 116:2399-408

9) Brooks, N.A., Grundy, J. E., Lavigne, N., Derry, M.C., Restall, C.M., MacKenzie, C.R., Waisman, D.E. and **Pryzdial, E.L.G.** (2002) Ca^{2+}-dependent, phospholipid-independent binding of annexin II to annexin V. Biochemical Journal 367:895-900

10) Zeibdawi, A. and **Pryzdial, E.L.G.** (2001) Mechanism of Factor Va inactivation by plasmin: Loss of A2 and A3 domains from a Ca^{2+}-dependnet complex of fragments bound to phospholipid. Journal of Biological Chemistry 276:19929-19936

11) Grundy, J., Hirama, T., MacKenzie, R. and **Pryzdial, E.L.G.** (2001) Binding of plasminogen and tissue plasminogen activator to plasmin-modulated coagulation factors X and Xa. Biochemistry 40:6293-6302

12) Raynor, C.M., Wright, J.F., Waisman, D.M. and **Pryzdial, E.L.G.** (1999) Annexin II enhances cytomegalovirus binding and fusion to phospholipid membranes. Biochemistry 38:5089-5095

13) **Pryzdial, E.L.G.**, Lavigne, N., Dupuis, N., Kessler, G.E. (1999) Plasmin converts factor X from coagulation zymogen to fibrinolysis cofactor. Journal of Biological Chemistry 274:8500-8505

14)Sutherland, M.R., Raynor, C.M., Leenknegt, H., Wright, J.F. and **Pryzdial, E.L.G.** (1997) Coagulation initiated on Herpesviruses. Proceedings of the National Academy of Sciences, USA 94:13510-13514

15)**Pryzdial, E.L.G.** and Kessler, G.E. (1996) Kinetics of blood coagulation factor Xaα autoproteolytic conversion to factor Xaβ: Effect on inhibition by antithrombin, prothrombinase assembly and enzyme activity. Journal of Biological Chemistry 271:16621-16626

16)**Pryzdial, E.L.G.** and Kessler, G.E. (1996) Autoproteolysis or plasmin-mediated cleavage of factor Xaα exposes a plasminogen binding site and inhibits coagulation. Journal of Biological Chemistry 271:16614-16620

17)**Pryzdial, E.L.G.**, Bajzar, L. and Nesheim, M.E. (1995) Prothrombinase components can accelerate tissue plasminogen activator-catalysed activation of plasminogen. Journal of Biological Chemistry, 270:17871-17877

18)Wright, J.F., Kurosky, A., **Pryzdial, E.L.G.** and Wasi, S. (1995) Host cellular annexin II is associated with cytomegalovirus isolated from cultured human fibroblasts. Journal of Virology, 69:4784-4791

19)**Pryzdial, E.L.G.** and Wright, J.F. (1994) Prothrombinase assembly on an enveloped virus: Evidence that the cytomegalovirus surface contains procoagulant phospholipid. Blood 84:3749-3757

20)**Pryzdial, E.L.G.** and Mann, K.G. (1991) The association of coagulation factor Xa and factor Va. Journal of Biological Chemistry 266:8969-8977

21)**Pryzdial, E.L.G.** and Isenman, D.E. (1988) A thermodynamic study of the interaction between complement components C3b or C3(H_2O) and factor B in solution. Journal of Biological Chemistry 263:1733-1738

22)**Pryzdial, E.L.G.** and Isenman, D.E. (1987) Alternative complement pathway activation fragment Ba binds to C3b: Evidence that formation of the factor B-C3b complex involves two discrete points of contact. Journal of Biological Chemistry 262:1519-1525

23)**Pryzdial, E.L.G.** and Isenman, D.E. (1986) A re-examination of the role of magnesium in the human alternative pathway of complement. Molecular Immunology 23:87-96

Papers in Refereed Conference Proceedings:

1) Talbot, K., Meixner, S.C. and **Pryzdial, E.L.G.** (2008) A Novel Blood Protein Application: Coagulation Factor Xa Becomes a Clot-Dissolving Agent. Transfusion 48(2S):141A

2) Krisinger, M.J., **Pryzdial, E.L.G.** and MacGillivray, E.L.G. (2007) A Comparison of the Membrane Binding Properties of the Gamma-Carboxy Glutamic Acid Proteins of Blood Coagulation. International Society of Thrombosis and Haemostasis

3) **Pryzdial, E.L.G.**, Meixner, S.C. and Talbot, K. (2007) Proteolytic Conversion of Clotting Factor Xa into a Fibrinolytic Accelerator. International Society of Thrombosis and Haemostasis

4) Hewitt, J., Talbot, K., Song, J. Ho, M. **Pryzdial, E.L.G.** and MacGillivray, R.T.M. (2007) Homozygous Factor V deficiency with mild clinical phenotype. International Society of Thrombosis and Haemostasis

5) **Pryzdial, E.L.G.**, Talbot, K. and Meixner, S.C. (2006) Plasmin-mediated coagulation FX derivative, Xa33/13, enhances plasminogen activation and fibrin clot lysis. American Association of Hematology

6) Derry, M.C., St-Pierre, S., Sutherland, M.R., Waisman, D.M. and **Pryzdial, E.L.G.** (2005) Annexin 1 and annexin 5 inhibit annexin 2-dependent cytomegalovirus infection. American Association of Blood Banks

7) Craven, S.J., Dewar, L., Hewett, J., Pryzdial, E.L.G., MacGillivray, R.T.A. and Ofosu, F.A. (2005) PLatelets provide factor IX and FX to support dilute tissue factor dependent coagulation. International Society of Thrombosis and Haemostasis

8) Krisinger, M.J., Pryzdial, E.L.G. and MacGillivray, R.T.A.The Effect of Factor Va on prothrombin-membrane binding. International Society of Thrombosis and Haemostasis

9) Sutherland, M.R., Friedman, H.R. and Pryzdial, E.L.G. (2004) Herpes Simplex Virus Infection of Endothelial Cells Is Enhanced by Thrombin. American Society of Hematology

10) Zeibdawi, A., Grundy, J.E., Lasia, B. and **Pryzdial, E.L.G.** (2003) Coagulation factor Va Glu96-Asp111: A chelator-sensitive site involved in function and subunit association. American Society of Hematology

11) Derry, M.C., Raynor, C.M., Waisman, D.M. and **Pryzdial, E.L.G.** (2003) Annexin II enhances cytomegalovirus Infection. International Annexin Workshop

12) **Pryzdial, E.L.G.,** Zeibdawi, A., Grundy, J.E. and Lasia, B. (2003) Coagulation factor Va Glu96-Asp111: A chelator-sensitive site involved in function and subunit association. Frontiers in Cardivascular Research

13)Sutherland, M.R., Friedman, H.R. and **Pryzdial, E.L.G.** (2002) Thrombin Enhances Herpesvirus Infection. American Society of Hematology

14)M.C. Derry, N.D. Brooks, J.E. Grundy, N. Lavigne, S. St-Pierre, C.M. Restall, C.R. MacKenzie, D.M. Waisman, and **E.L.G. Pryzdial** (2002) Annexin 2-Annexin 5 Interaction: Implications for Cytomegalovirus Infection. International Society of Blood Transfusion

15)Sutherland M.R. and **Pryzdial E.L.G**. (2002) Comparison of the Direct Thrombogenic Risk Of Cytomegalovirus and Herpes Simplex Virus Types 1 and 2. International Society of Blood Transfusion

16)Derry, M.C., Raynor, C.M., Waisman, D.M. and **Pryzdial, E.L.G.** (2001) Annexin II in cytomegalovirus Infection. International Herpesvirus Workshop (Germany)

17)Grundy, J.E., Hancock, M.A., MacKenzie, C.R., Koschinsky, M. and **Pryzdial, E.L.G.** (2001) Calcium- and C-terminal lysine-dependent interactions between plasminogen and plasmin-modulated factor Xa. International Society of Thrombosis and Haemostasis

18)Sutherland, M.E., Friedman, H., Cohen, G., Eisenberg, R. and **Pryzdial, E.L.G.** (2001) Herpes simplex virus glycoprotein C mimics tissue factor. International Society of Thrombosis and Haemostasis

19)Peterson, E., Sutherland, M. and **Pryzdial, E.L.G.** (2001) Effect of thrombin on annexin II cellular distribution. Canadian Federation of Biological Sciences

20)Derry, M.C., Raynor, C.M., Waisman, D.M. and **Pryzdial, E.L.G.** (2001) Annexin II in cytomegalovirus Infection. Canadian Federation of Biological Sciences

21)Zeibdawi, A., Hu, J., Peterson, E., Bazin, R., Aye, M.T., Lemieux, R., Giulivi, A., **Pryzdial, E.L.G.** (2001) A novel C2 domain-containing protein, P110, is blood cell specific and binds anionic phospholipid. Canadian Federation of Biological Sciences

22)Sutherland, M.R., Friedman, H., Cohen, G., Eisenberg, R. and **Pryzdial, E.L.G.** (2001) Herpes simplex virus glycoprotein C mimics tissue factor. Canadian Federation of Biological Sciences

23)Grundy, J.E., Hancock, M.A., MacKenzie, C.R., Koschinsky, M. and **Pryzdial, E.L.G.** (2001) Calcium- and C-terminal lysine-dependent interactions between plasminogen and plasmin-modulated factor Xa. Canadian Federation of Biological Sciences

24)Zeibdawi, A. and **Pryzdial, E.L.G.** (2001) Regulation of coagulation factor Va. Canadian Society of Transfusion Medicine

25)Grundy, J. E., Hancock, M.A., Mackenzie, C.R. Koschinsky, M. and **Pryzdial, E.L.G.** (2001) Dissecting a new function for coagulation factor Xa. Canadian Society of Transfusion Medicine

26)Sutherland, M.R., Gillies, C., Friedman, H., Cohen, G., Eisenberg, R. and **Pryzdial, E.L.G.** (2000) Herpes Simplex Virus Glycoprotein C Links Infection to Coagulation, American Society of Blood Bankers

27)Derry, M.C., St-Pierre, S., Raynor, C.M., Waisman, D., **Pryzdial, E.L.G.** (2000) Annexins in cytomegalovirus infection. Canadian Society of Transfusion Medicine

28)Raynor, C.M., Krzykwa, E., Neron, S. Lemieux, R. and **Pryzdial, E.L.G.** (2000) Apoptosis and necrosis of hybridoma cells are inhibited by BCL-xl. Canadian Society of Transfusion Medicine

29)Grundy, J., Hirama, T., MacKenzie, R. and **Pryzdial, E.L.G.** (2000) Binding of plasminogen and tissue plasminogen activator to plasmin-cleaved coagulation factors X and Xa. Canadian Society of Biological Sciences

30)Sutherland, M.R., Gillies, C., Friedman, H., Cohen, G., Eisenberg, R. and **Pryzdial, E.L.G.** (2000) Herpes simplex virus type-1 glycoprotein C initiates coagulation. International Herpesvirus Workshop

31)Zeibdawi, A. and **Pryzdial, E.L.G.** (2000) Coagulation factor Va-derived fibrinolysis cofactor. Canadian Society of Biological Sciences

32)Peterson, E., Sutherland, M.R. and **Pryzdial, E.L.G.** (2000) Effect of Thrombin on cellular annexin II distribution. Canadian Society of Biological Sciences

33)**Pryzdial, E.L.G.**, Raynor, C.M., Wright, J.F. and Waisman, D.M. (1999) Annexin II enhances cytomegalovirus binding and fusion to phospholipid membranes. American Society of Virology

34)**Pryzdial, E.L.G.**, Lavigne, N., Dupuis, N., Kessler, G.E. (1998) Modulation of coagulation zymogen factor X by the fibrinolysis enzyme plasmin. Gordon Research Conference on Hemostasis

35)**Pryzdial, E.L.G.**, Barrette, N., Dupuis,N. and Kessler, G.E. (1998) Regulation of coagulation factor X by the fibrinolysis enzyme plasmin. Transfusion Medicine

36)Raynor, C.M., Néron,S., Lemieux, R. and **Pryzdial, E.L.G.** (1998) Early detection of hybridoma cell apoptosis. Transfusion Medicine

37)Brooks, N.D., Raynor, C.M. and **Pryzdial, E.L.G.** (1998) Interaction between annexin II and annexin V: Implications for cytomegalovirus infection. Transfusion Medicine

38)Zeibdawi, A., Hu, J., **Pryzdial, E.L.G.**, Lemieux, R., Aye, M.T. and Giulivi, A. (1998) A novel C2 domain-containing protein, p110, is blood cell specific and binds to anionic phospholipid. Transfusion Medicine

39)**Pryzdial, E.L.G.**, Raynor, C.M., Sutherland, M.R., Wright, J.F. (1997) Cytomegalovirus enhances host annexin II exposure: Direct role in membrane binding and cell infection. American Society of Virology 16th Annual Meeting Proceedings: 230.

40)Sutherland, M.R., Raynor, C.M., Wright, J.F. and **Pryzdial, E.L.G.** (1997) Viral Activation of factor X: Evidence for tissue factor on the surface of cytomegalovirus, herpes simplex virus-1 and -2. Thrombosis and Haemostasis, Supplement

41)Raynor, C.M., Sutherland, M.R., Wright, J.F. and **Pryzdial, E.L.G.** (1997) Purified annexin II is a cytomegalovirus receptor: up-regulation on the cell surface by virus. Transfusion Medicine

42)Pryzdial, E.L.G., Kessler, G.E. (1996) Autoproteolysis or plasmin-mediated cleavage of factor Xa-alpha exposes a plasminogen binding site and inhibits coagulation. Gordon Research Conference on Hemostasis

43)**Pryzdial, E.L.G.** and Kessler, G.E. (1996) The modulation of clotting factor Xa function by autoproteolysis and plasmin. Transfusion Medicine 7:158

44)Sutherland, M.R., Raynor, C.M., Wright, J.F. and **Pryzdial, E.L.G.** (1996) Procoagulant mechanisms of *Herpesviridae.* Transfusion Medicine 7:163

45)**Pryzdial, E.L.G.** (1995) Feedback conversion of prothrombinase into a tissue plasminogen activator-accelerator and plasminogen receptor. Thrombosis and Haemostasis, 73:1129

46)**Pryzdial, E.L.G.** (1995) Plasmin-mediated conversion of coagulation factors Xa and Va into fibrinolytic cofactors. Transfusion Medicine 6:83

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APPENDIX B

Heart and Stroke Foundation of Canada

Scientific Review Committee Report
1999/2000 Funding Year

Applicant: Pryzdial, Edward L. **Foundation:** Ont.
Co-applicant: **Review Committee:** VI
Co-applicant:
Application Code: G-99-PR-0277
Title: *A novel fibrinolysis accelerator localized by procoagulant phospholipid.*

Committee Discussion:

This is a novel proposal with potentially important therapeutic applications that is based on exciting preliminary work by the applicant. Appropriate collaboration for sophisticated protein chemistry and structural studies has been arranged.

The clinical trial (part III) is not well described, and important details related to this section are missing. Productivity has been very good. One of the externals questioned the physiologic relevance of studies done in the absence of fibrin, but the committee felt that overall there was sufficient solid biochemistry to place the grant in the very good to excellent range.

HSFC 1999/2000 Grant Application Review
Application Type GIA - ER1 - Ont.
Application #G-99-PR-2277
Title "A novel fibrinolysis accelerator localized by procoagulant phospholipid: Plasmin-mediated conversion of the coagulation enzyme complex, prothrombinase, into a tissue plasminogen activator cofactor"
Applicant Edward L.G. Pryzdial
New Grant (3 yrs) 1999/2000 $73 101
2000/2001 $71 013
2001/2002 $71 441

Applicant:
The applicant completed his post-doc at the University of Vermont and subsequently moved to The Canadian Red Cross Society as a Research Scientist in 1990. More recently, 1996, Dr. Pryzdial became a Senior Research Scientist. During this time he also received a faculty appointment as an Adjunct Professor in the department of Biochemistry, Microbiology and Immunology at the University of Ottawa.

Research Environment:
Both the research environment and collaborations are considered Excellent.

Other Funding:
Three grants from the Canadian Red Cross R&D Fund, 1 equipment grant also from the CRCRD as well as a grant from the MRC. No obvious overlap is apparent.

Progress:
This grant would, in essence, facilitate continuance of work funded, until October of this year, by the Canadian Red Cross R&D Fund. A paper describing preliminary work of significance to this grant has been submitted to J. Biol. Chem. Dr. Pryzdial has published at least one paper per year in very good quality journals. Furthermore, recent work appears promising.

Budget:
The previous CRCRD grant was approximately $16 000 less than that requested here. Cloning factor V (probably premature), travel money request ($2500), and student would contribute to this increase.

Summary, Critique and Suggestions:
Conversion of components of prothrombinase, in the presence of procoagulant phospholipid, from a procoagulant into a multimeric stimulator of tPA-induced fibrinolysis by plasmin comprises a novel link between coagulation and fibrinolysis. In order to understand this the applicant has proposed to; 1) investigate the molecular basis for the effects of plasmin on prothrombinase by determining the plasmin catalyzed cleavage sites on both FVa and FX; 2) quantify plasmin cleaved prothrombinase-derived cofactor activity in terms of catalyzing the rate of tPA-dependent plasminogen activation and subsequently determining various binding

constants; and 3) to identify plasmin-mediated prothrombinase and FX fragments by western blotting of samples produced *in vitro* from lysing(ed) clots formed from purified components, plasma, platelet rich plasma and whole blood.
The applicant has uncovered a previously unknown link between coagulation and fibrinolysis which has potential clinical relevance. These observations are unique and should be pursued. The proposed experiments are feasible and the applicants choice of collaborators is of considerable benefit.

Of concern, however, is that the observations were made in the absence of fibrin. The applicant must demonstrate that plasmin catalyzed cleavage of prothrombinase components enhance tPA-mediated fibrinolysis not just tPA-dependent activation of plasminogen in solution. This could be done with purified components or plasma depleted of various components. The results would be biased toward success for two reasons. First, the plasmin treated components would be in place prior to clot formation and thus would be incorporated into the clot during fibrinolysis. Second, cleavage of these components by plasmin generated *in situ* would not be a requirement. Positive results would indicate whether measurement of plasmin cleaved FX/Xa/V/Va products in plasma is relevant. If an effect is observed the concentration-dependence of this effect will either substantiate or refute the applicant's hypothesis that these components (or analogues thereof) would be clinically advantageous. Since the concentration of FV or even that of FX in plasma is at least an order of magnitude less than the concentration of fibrinogen (fibrin), a ratio which would probably be maintained within a clot, it is difficult to envisage a scenario where components of prothrombinase will be cleaved by plasmin in sufficient quantity to compete with fibrin (both as a substrate for plasmin and a cofactor for tPA) to stimulate tPA-dependent activation of plasminogen. Subsequently, the applicant must demonstrate that, not only are the components produced during fibrinolysis (specific aim 6.3.1), but that they are produced in a time-course that would facilitate tPA-mediated fibrinolysis. It must also be noted that a clinically relevant drug would have to gain access to a preformed thrombus. The relevance of this project's long term goals is in question until these experiments are performed.

The applicant has initiated the cloning of FV in an attempt to produce enough FV to produce antigen for mAb and generation of recombinant tPA cofactors. Two other laboratories have published on the cloning and expression of FV. There are no convincing evidence that more FV could be produced in this fashion than isolated from plasma. Sequencing of fragments and subsequent production of synthetic peptides may be more effective/productive.

Presumably consent forms will be required from patient participating in Specific Aim 6.3.2. "Plasma from patients receiving thrombolytic therapy". A human research form should probably be included.

Assessment and Recommendations:
In general this proposal is well written. All the experiments proposed appear feasible and potentially will yield useful data. The caveat is that the effect of plasmin cleaved prothrombinase components on tPA-mediated fibrinolysis has not been assessed and their stimulatory effect may not be observed in the presence of fibrin. Although, relevance is in question it should not preclude investigation of this effect since the magnitude of this effect appears so large. The subject matter is intriguing and requires investigation. The grant should be funded providing the subject of "relevance" is addressed during its tenure.

Title: A Novel Fibrinolysis Accelerator Localized by Procoagulant Phospholipid: Plasmin-Mediated Conversion of the Coagulation Complex, Prothrombinase, into a Tissue Plasminogen Activator Cofactor.

Applicant: Dr. Edward L.G. Pryzdial

Summary: The proposed work is directed at studying a proposed link between coagulation and fibrinolysis whereby the prothrombinase complex is modified by plasmin to develop tPA-cofactor activity. The long-range goal is to provide a clot specific cofactor for tPA that can be used therapeutically. The hypothesis of the proposal is that plasmin converts prothrombinase into multimeric cofactors for tPA. Three specific objectives are proposed to test this hypothesis. These are: (1) to determine the molecular basis for the effects of plasmin in prothrombinase, (2) to quantify prothrombinase derived tPA cofactor activity, and (3) to identify plasmin mediated prothrombinase and factor X fragments in biological systems. These objectives will be accomplished through experiments that utilize standard techniques of protein chemistry and enzymology.

Assessment: The strengths of the proposal are that its hypothesis is solidly based on previous work, it deals with a highly relevant and timely topic, and the applicant has been productive and has demonstrated, through high quality publications, the ability to extract definitive, quantitative new information from this intrinsically complex, multicomponent system. A weakness of the proposal is that it does not go very far in exploring the question of whether the phenomenon observed *in vitro* (enhancement of plasminogen activation by plasmin-modified prothrombinase components) is physiologically relevant or an epi-phenomenon. Because plasminogen activation *in vivo* presumably would occur primarily within a clot, overwhelmingly high levels of fibrin, relative to those of plasmin-modified prothrombinase components, would be available to serve the tPA cofactor function. Relatively straight-forward experiments whereby lysis of fibrin by tPA plus plasminogen, in the presence or absence of prothrombinase components, are needed to determine whether the latter substantially contribute to the kinetics of fibrinolysis. In addition, the concept of directing fibrinolytic cofactors to a site expressing procoagulant activity might be difficult to justify logically if one of the goals is to avoid bleeding. Nonetheless, the work proposed here, if successfully executed, should provide a thorough characterization of plasminogen activation by plasmin-modified prothrombinase components, regardless of whether the phenomenon turns out to have physiologic relevance.

Rating: This is a very good proposal from an excellent investigator (3.9)

APPENDIX C

BIOCHEMISTRY

Manuscript No. → bi00 2200 V-1-0-0 — Reviewer No.

Corresponding → Author: J. C. Gruney, N. Lavigne, T. Hirama, C. Rogerf, Mackenzie, Edward L.G. Przydial

Abbreviated → Title: Binding of Plasminogen and Tissue Plasminogen Activator To Plasmin-Modulated Factor X and Factor Xa

Ms. Type: a:article

Comments:

This manuscript details some biochemical background information on the previously observed stimulation of tPA-induced plasminogen activation by plasmin-degraded factor X and factor Xa. The experiments look, in general, solid and the manuscript is well written. My major criticism concerns not particularly this manuscripts, but all papers in this series (see Ref 5-7) which all deal with experiments in purified systems. I wonder whether degradation of Factor X(a) by plasmin really occurs in a plasma milieu, in particular in the first phase of clot lysis as suggested on page 24, second paragraph. In addition I wonder whether or not the stimulatory potential of degraded FX(a) on tPA-induced palsminogen activation is relevant in the presence of a great stimulatory potential of intact fibrin in the first phase of clot lysis and of partially degraded fibrin in the second phase of clot lysis. I would recommend the authors to concentrate first on the physiological relevance of the proposed mechanism before to continue with new biochemical studies.

Comments specific for this manuscript:

Experimental Procedures

1. Page 9, line 12/13: Indicate the details of the buffer used and clearly state when calcium is present or absent during the incubation with radiolabeled tPA.
2. Page 11, formulae 4 and 5 are, in my opinion, wrong: they contain (in total) three times a factor 2 which should not be present. I cannot see if deletion of these factors does affect the rest of the analysis.
3. Page 12, formula 6: just a question, I believe that this formula is valid in a univalent model, but I do not know if this formula is also valid in a bivalent model.

Results

4. I am not 100% convinced by the ligand blot experiments with tPA. It is my personal experience that tPA can bind to almost all proteins in this type of binding studies. Serious control experiments are therefore required.
5. Because of my concern about tPA binding demonstrated by ligand blotting (comment 4), it is a pity that the authors did not study tPA binding in SPR as extensively as plasminogen binding.
6. I do not fully understand Figure 8, in particular the relationship between panel A and panel B. The RU at the highest plasminogen concentrations seems to be lower in panel B than in panel A. The text on page 18 (line 7) speaks about "at the end of injection". How long was the injection?

Discussion

7. Page 21, last paragraph: some figures (0.5 nM in line 19 and 5-fold in line 20) are apparently not compatible with Table 1.

References

8. Ref. 32 is from 1986